Pilot-Scale Semisolid Fermentation of Straw†

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Semisolid fermentation of ryegrass straw to increase its animal feed value was successfully performed on a pilot scale. The pilot plant, which could handle 100 kg of straw per batch, was designed so that all major operations could take place in one vessel. The straw was hydrolyzed at 121°C for 30 min with 0.5 N H2SO4 (7:3 liquid:solid), treated with ammonia to raise the pH to 5.0, inoculated with Candida utilis, and fermented in a semisolid state (70% moisture). During fermentation the straw was held stationary with air blown up through it. Batch fermentation times were 12 to 29 h. Semisolid fermentation did not require agitation and supported abundant growth at 20 to 40°C even at near zero oxygen tensions. Fermentation increased the protein content, crude fat content, and in vitro rumen digestibility of the straw.

The production of single-cell protein from waste cellulosics has been suggested as a means of using them, but as yet has not proven economically practical (1). Semisolid fermentation may reduce the cost of growing microorganisms on cellulosic wastes, making their utilization more feasible. We have been investigating increasing the feed value of ryegrass straw by using semisolid fermentation to increase the in vitro rumen digestibility and protein and fat contents of the straw (4, 9). In vitro tests look promising, but the true feed value of fermented straw must be determined by in vivo trials. Several hundred kilograms of fermented straw must be produced for feeding and metabolism studies on sheep or cattle. To produce this amount of fermented straw and to study the characteristics of semisolid fermentation of straw on a larger scale, a pilot-scale hydrolysis-fermentation plant was constructed. This paper describes the plant design and operation and the characteristics of the fermented product.

MATERIALS AND METHODS

Microorganism. Candida utilis (NRRL Y-1084) was used as the test organism. It was grown and maintained as described by Han and Anderson (4). The inoculum for the first pilot plant fermentation was prepared in a New Brunswick Microferm laboratory fermentor by using 10 liters of liquid straw hydrolysate.

Substrate. Perennial ryegrass (Lolium perenne Lamb.) sun dried and hammer milled to pass a 6.4-mm (0.25-inch) screen, was used as the substrate. For laboratory hydrolyses, one part of straw was mixed with three parts of 0.5 N H2SO4 and heated at 121°C for 30 min in an autoclave. The mixture was adjusted to pH 4.5 to 5.0 with NH4OH. To prepare liquid hydrolysate, the mixture was squeezed in a wine press, and the liquid was diluted to a reducing sugar content of 3%. For pilot plant work, the straw was hydrolyzed as described below.

Fermentation. Screw-capped, approximately 1-liter jars were used for small-scale fermentations. The bottles were partially filled with acid-hydrolyzed and ammoniated straw and were held perpendicular to a board rotating vertically at 0.5 rpm. Actively growing cultures were mixed with the straw (10%, vol/wt), and the fermentation was continued for 3 days.

Analytical procedures. Total nitrogen was determined by the micro-Kjeldahl method of Perrin (7). NH3-N was determined by the method of Jackson (6). Moisture was determined by drying to a constant weight at 105°C. The pH was measured on samples mixed (1:10) in distilled water. Microbial counts were made from 1-g samples blended for 30 s in 100 ml of distilled water. Samples were serially diluted and plated on potato-dextrose agar.

Sugars were determined with a gas chromatograph. Straw samples were soaked in distilled water (1:10, wt/vol) and filtered through Whatman no. 1 filter paper. The filtrate was lyophilized, and 20 to 30 mg of the lyophilized material was dissolved in 1 ml of Tri-Sil Z (Fierce Chemical Co.). The silylated sugars were injected on a column of General Electric SE 30 silicone on Anakrom diatomaceous earth (Analabs, Inc.). Column temperature was 190°C.

In vitro rumen digestibility was determined by the modified method of Mellenberger et al. (6). A 0.5-g amount of substrate and 35 ml of rumen fluid were placed in a 50-ml screw-capped bottle and incubated for 2 to 3 days at 39°C. The digest was filtered through a sintered-glass crucible, and the residue was dried overnight at 105°C. Weight loss is reported as percentage of in vitro rumen digestibility.
The CO₂ concentration in the air from the pilot plant fermentations was monitored with a Mine Safety Appliances Co. Lira infrared analyzer.

Cellulose, hemicellulose, lignin, and ash were determined by the method of Goering and Van Soest (2).

RESULTS

Plant design and operations. Figure 1 is a view of the hydrolysis fermentation plant. The principal piece of equipment in the plant is a 2,270-liter (600-gallon), stainless steel-lined, jacketed pressure vessel. This vessel was formerly used for preparing urea-formaldehyde resins. It was modified so that straw hydrolysis, neutralization, fermentation, and drying could all be performed inside it. Two mixing paddles running the length of the tank revolve at 6 rpm and can completely mix the contents of the tank in 45 min. The paddles are canted slightly so that straw is moved horizontally as well as turned over. The mixer drive can handle up to 330 kg of 70% moisture straw.

The steps in a typical hydrolysis-fermentation run in the pilot plant are diagrammed in Fig. 2. Straw, hammer milled to pass a 6.4-mm (0.25-inch) screen, was obtained from the Oregon Field Sanitation Committee. An air flow of 25 m³/min from a 7.46-kW (10-horsepower) blower moved the straw from a pallet box into the pressure vessel through a 15.2-cm (6-inch)-diameter hose. Water and acid were mixed in a plastic tank and pumped into the main vessel through a polyvinylchloride pipe. The volume of water added plus the steam condensate produced during hydrolysis gave a moisture content of 70% in the hydrolyzed product. The acid concentration was 2.5% (0.5 N) on a weight of acid to volume of water basis or 5.8% on a weight of acid to dry weight of straw basis.

A 5.08-cm (2-inch), 4.5 × 10⁻⁵ N/m² gauge pressure (60-psig) steam line supplied heat to the pressure vessel. Steam was injected directly into the interior of the vessel during hydrolysis. An air exhaust valve was left open until the interior temperature reached 100°C. The steam raised the interior temperature from 20°C to 121°C in 15 min. The temperature was held constant at 121°C for 30 min on most runs. The mixing paddles continued to revolve throughout the hydrolysis and cooling operations and the addition of ammonia and inoculum. After hydrolysis, the steam was exhausted through an aspirator to dissolve the sulfuric acid fumes and hot steam in a cold water stream. The tank dropped to atmospheric pressure in 4 min. The tank was further cooled by blowing dry air through it with the 7.46-kW fan. This evaporative cooling lowered the temperature of the straw to 30°C in 30 min. To adjust the pH of the straw to 5.0, the tank was sealed, and ammonia gas was fed into it. The amount of ammonia added was determined from the decrease in weight of the ammonia cylinder.

A 30-kg amount of wet, fermented straw saved from the previous fermentation and kept at 4°C was mixed with the hydrolyzed, ammonia-treated straw to give it a 10% inoculum of C. utilis. For fermentation the mixers were stopped, and a 5-cm (2-inch)-diameter pipe was buried under the straw as shown in step 4 of Fig. 2. The pipe had 0.64-cm (¼-inch)-diameter holes drilled at 5-cm spacings along its length. One end was sealed, and the other was attached to a Nash Hy-Torr brand pump. The pump supplied 1.7 m³ of air per min, which percolated up through the bed of fermenting straw. The Hy-Torr pump used a water seal that saturated the

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Fig. 1. View of the straw hydrolysis-fermentation pilot plant.

Fig. 2. Processing steps in straw hydrolysis and fermentation in the pilot plant.
air with water vapor, so it caused no evaporation from the fermenting mass. The fermenting straw formed a pile approximately 33 cm high by 90 cm wide by 162 cm long in the tank.

When the CO₂ concentration in the fermentor passed its maximum level and began to decline, which occurred 12 to 29 h after inoculation, the aeration pipe was removed, samples were taken for plate counts, and 30 kg of straw was saved in a plastic sack as an inoculum for the next batch. The straw was dried to 10% moisture by dry air blown through the tank with the mixers operating and steam supplied into the tank jacket at a gauge pressure of 2.77 x 10⁵ N/m² (40 psig). The drying process required 16 h. The dry, fermented straw was sucked out of the vessel and blown into a pallet box with the same equipment used in loading the vessel.

Cell growth. C. utilis grew well on the hydrolyzed straw. Starting with 1 x 10⁹ cells per dry gram of straw, the concentration was 1 x 10⁹ cells/dry gram at the end of fermentation. The increase was similar to that observed in laboratory fermentations. A semilog plot of the CO₂ concentration in the air coming off the fermenting straw is shown in Fig. 3. By using the slope of the linear portion of this curve, the yeast doubling time was calculated. The average doubling time was 3.7 ± 1.0 h. Fermentation times ranged from 12 to 29 h. This wide range of fermentation times may be due to variations in the viability of the inocula. During the first two pilot plant runs, a small amount of bacterial contamination was noted when cell counts were made. During later runs, even this small contamination was not observed.

Fermentor configuration. In the first attempts to ferment straw in the pilot plant, the mixers in the vessel were left on and air was blown through the tank. All attempts to ferment the material with the mixers on were unsuccessful. Cell counts and CO₂ recordings rapidly declined in the first 16 h after inoculation, then slowly increased, and peaked out after 4 or 5 days at about 1 x 10⁹ cells per dry gram of straw. Several designs had been successfully used for laboratory-scale, semisolid fermentation. These included mason jars rotated at 0.5 rpm, a 5-cm-diameter by 30-cm-high column of straw with air blown up through it from the bottom, and a 100-liter cement mixer rotated at 0.5 rpm. In all these laboratory fermentors, the agitation was less severe than in the big vessel with the mixers operating. As an experiment, the pilot plant mixers were turned off after mixing in the inoculum, a perforated pipe was buried under the straw, and air was blown through the pipe. This arrangement produced a successful fermentation and was used in all subsequent fermentations. Apparently, yeast growth was inhibited by the severe agitation in earlier runs.

Suspecting that oxygen transfer would be insufficient in a pile of straw with one aeration pipe in the center, we experimented with a 190-liter (50-gallon) barrel rigged with a screen elevated 9 cm above the bottom. Air was blown in below the screen and flowed upward through a bed of straw 30 cm thick. Aeration was definitely better in the barrel than in the pilot plant vessel. An oxygen probe buried in the straw in the barrel at several locations showed that oxygen concentrations at peak fermentation varied from 7.7% (mole fraction) just above the screen to 19.5% near the top of the straw. Similar measurements on the pile of fermenting straw in the pilot plant fermentor showed no measurable oxygen in most of the pile. In the region directly above the aeration pipe, however, oxygen concentrations of 18% (mole fraction) were recorded. Temperatures in the barrel ranged from 27 to 30°C. Temperatures in the pilot plant fermentor were 26 to 29°C directly above the aeration pipe but were as high as 45°C in other locations. Temperatures over 35°C were recorded in large portions of the fermenting straw. These readings suggest that insufficient air to provide oxygen for growth and to remove excess heat was supplied to much of the pile in the large fermentor. Cell counts, however, did not correlate with oxygen concentration and temperature. Cell counts on samples from several locations having different O₂ concentrations and temperatures were not significantly (P = 0.5) different. Growth rates determined from CO₂ evolution were identical for the barrel and the pilot plant fermentor.

An oxygen probe buried in the fermenting
straw does not directly measure the oxygen concentration in the microenvironment of the cell. The semisolid mass, unlike a liquid, has air spaces between the particles. The probe measured the oxygen concentration in the air spaces, not the oxygen dissolved in the liquid adsorbed on the straw particle. The yeast cells must depend on dissolved oxygen in the adsorbed liquid. Because diffusion of oxygen is from the air pockets into the water on the straw particles, the oxygen levels measured by the probe indicated the upper limits of the oxygen concentrations in the microenvironments of the cells. Because oxygen probe readings in the pilot plant fermentor were zero at most locations, most of the cells in the fermenting straw must have actually been exposed to oxygen concentrations below the limit of detection of the probe (about 0.15% O₂, mole fraction).

Although C. utilis will grow under anaerobic conditions, its growth rate is much slower than under aerobic conditions. It is more likely that enough oxygen diffused into the straw to support aerobic yeast growth, although perhaps at a lower than optimal rate and that oxygen was consumed as rapidly as it diffused inward.

**Chemical composition and digestibility.** Table 1 gives some characteristics of the pilot plant-fermented straw, untreated straw, and laboratory-fermented straw. The fermented straws have a higher cell soluble matter content and a lower hemicellulose content than untreated straw. These changes were due to solubilization of the hemicellulose during hydrolysis. The total Kjeldahl nitrogen content of laboratory-fermented straw is higher than that of pilot plant-fermented straw because different amounts of ammonia were added due to different hydrolysis conditions. The laboratory straw was hydrolyzed at a liquid-to-solid ratio of 3:1 and, thus, required more ammonia than the pilot plant straw, which was hydrolyzed at a 7:3 ratio. Less liquid was used with the pilot plant straw because it formed mud-like clumps at moisture contents of 75% or greater.

**Sugar utilization.** The amounts and kinds of sugars released during acid hydrolysis depend on the acid concentration, temperature, hydrolysis time, and liquid-to-solid ratio (3). The hydrolysis conditions used in the pilot plant (seven parts 0.5 N H₂SO₄ to three parts straw at 121°C for 30 min) were shown in previous laboratory work (4) to release adequate levels of sugar for maximum yeast growth. Gas chromatographic analyses of samples from the pilot plant showed that these hydrolysis conditions produced 3.3 g of mannose, 2.0 g of xylose, and 0.10 g of glucose per 100 g of dry straw. The yeast utilized the mannose and glucose completely, but did not change the xylose concentration. C. utilis is reported to utilize xylose but often weakly (8).

**DISCUSSION**

One of the first observed differences between the pilot plant and laboratory-fermented straws was their texture. Laboratory hydrolyses were carried out with small amounts of straw held in jars without agitation. After hydrolysis the jars were moved to a wheel rotating at 0.5 rpm for fermentation. During hydrolysis in the pilot plant, the straw was continuously mixed. The laboratory-hydrolyzed straw had the same physical appearance after hydrolysis as before. The pilot plant-hydrolyzed material had a much larger proportion of fine particles and a greater tendency to stick together in clumps than the material before hydrolysis. Evidently, the combination of acid, heat, and mixing in the pilot plant acted to grind the straw. The pilot plant-fermented straw showed a greater increase in in vitro rumen digestibility than laboratory-fermented straw, perhaps because of the greater proportion of finely ground material in the pilot plant product.

Moisture content during hydrolysis was critical. At a 70% moisture level, the straw was friable and packed loosely when piled. At moisture levels of 75% or greater, pilot plant-hydrolyzed straw became mudlike and stuck together in large clumps that were difficult to mix or aerate. Previous laboratory work indicated that microbial growth on straw was inhibited at moisture levels below 66% (9).

Abundant yeast growth in spite of the low oxygen tensions and high temperatures measured suggests that large-scale semisolid fermentation is simpler than submerged liquid fermentations. Unlike liquid fermentations, agitation was unnecessary and was in fact detrimental to

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**Table 1. Chemical composition and digestibility of untreated and fermented straws**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% of dry matter</th>
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<tbody>
<tr>
<td></td>
<td>Untreated straw</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Lignin</td>
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<td>Ash</td>
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<td>Ammonia nitrogen</td>
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<tr>
<td>IVRD</td>
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</tbody>
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* IVRD, In vitro rumen digestibility.
yeast growth. We speculate that the rubbing action caused by the tumbling straw knocked the yeast cells away from the straw particles and thus away from their substrate (i.e., sugar dissolved in the liquid adsorbed on the particles). Microscopic observation of successfully fermented straw showed yeast cells surrounding the straw particles. The few yeast cells observed in samples from mixed straw were not near straw particles.

Fermented straw was used as an inoculum for the next fermentation for ten cycles with no observed contamination or decrease in viability of the culture. The combined effects of low pH, limited nutrient, and short fermentation time prevented contamination.

The dried product was considerably finer than ground straw due to the grinding action of the mixers. Dried, fermented straw had a bulk density of 240 kg/m³ as compared to a bulk density of 130 kg/m³ for untreated, ground straw. The higher bulk density of fermented straw should be advantageous for handling and transporting. Unlike untreated straw, fermented straw could be moistened and pelleted easily. The fermented straw pellets produced in one trial run were quite hard. It may be necessary to adjust the pelleting conditions or mix in other ingredients to achieve a pellet palatable to animals. When dried to a 10% moisture level, the fermented straw showed no tendency to absorb moisture or mold during storage in a covered area for 6 months. Although animal feeding data are not yet available, the in vitro data in Table 1 suggest that the feed value of fermented straw will be appreciably greater than that of untreated straw.

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LITERATURE CITED